VERSION WITH MARKINGS TO SHOW CHANGES MADE IN THE CLAIMS

The changes relative to the previous version of the rewritten claim 28 are marked up as follows.

Claim 28 (amended). A preparation method of immunoglobulin Y (IgY) against dental caries bacteria, including the steps of:

(a) preparing streptococcus mutans antigen as antigen bacteria by the steps of;

(a1) separately cultivating said streptococcus mutans type c and type d in a culture medium for 2 to 3 days;

(a2) collecting bacteria by centrifugation;

(a3) washing said bacteria 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes;

(a4) mixing said streptococcus mutans type c and type d in a ratio of 2:1; and

(a5) adding Freund's adjuvant equal to total volume of said streptococcus mutans type c and type d with high speed homogenized;

(b) immunizing hens with said streptococcus mutans antigen to obtain eggs with active antibody from said hens [for 13 months];

(c) extracting a crude IgY from said eggs by water dilution method;

(d) applying said crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer containing 0.07M of NaCl to obtain eluates of protein peak;

(e) applying said eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain new eluates of protein peak;

(f) collecting [the] said new eluates of protein peak;

(g) estimating antibody activity of [the] said eluates of protein peaks with "ELISA"; and

(h) eliminating bacteria by 0.22μm membrane filtration and lyophilizing to achieve a purified IgY against dental caries bacteria.

REMARKS-General

1. The independent claim 28 is amended to delete the new matter "for 13 months" introduced in the Amendment B to overcome the rejection under 35USC112, first paragraph. No new matter has been included. All claims 28 to 33 are submitted to be of sufficient clarity and detail to enable a person of average skill in the art to make and use the instant invention, so as to be pursuant to 35 USC 112.

Response to Rejection of Claim 31 under 35USC112, second paragraph

2. The applicant submits that the original claim 31 is depended on claim 29 which is a dependent claim depends on claim 28, wherein although the dependent claim 29 claims the step (b) comprising steps (b1) to (b3), since the dependent claim 29 depends on claim 28, the claim 29 includes all the recitation of the independent claim 28, so that the antecedent basis of the step (c) as recited in claim 31 is supports in claim 28 which is depended by the claim 29. Therefore, the original claim 31 particularly points out and distinctly claims the subject matter of the instant invention, as pursuant to 35USC112, second paragraph.

Response to Rejection of Claims 28 to 33 under 35USC103

3. The Examiner rejected claims 28-33 as being unpatentable over Chang et al in the view of US Pat No. 4,4324,782, US Pat No. 5,367,054, and Akita et al.

4. The applicants respectfully submit an attachment table in order to draw comparisons of the claims of the present invention vs. the cited arts one by one.

5. Regarding to claim 28(a1), 31(a)

Chang et al teach cultivating c for **18 hours**, not **2 to 3 days** of the present invention, the time of cultivating is different. The '782 patent teach cultivating S. mutans for 48 hours, not make out c and d and the other cited arts do not teach this step.

6. Regarding to claim 28(a2), 31(b)

Chang et al teach to 'treat with **0.5% formalin** for 24 h'; the present invention need not to treat with formalin. Similar with '782 patent, but the subject matter of '782 patent is to immunize cow, not hens. The other cited arts do not teach this step.

7. Regarding to claim 28(a3), 31(c)

Chang et al teach to wash with sterile saline **containing 0.5% formalin.** The present invention is washing with phosphate buffered saline, which **containing no formalin**, and then **heating at 50-60°C** for 25 to 35 minutes. Similar with '782 patent, but the subject matter of '782 patent is to immunize cow, not hens. The other cited arts do not teach this step.

8. Regarding to claim 28(a4), 31(d)

Chang et al teach 70% of human dental caries caused by c, and suggest apply c and d simultaneously, **do not make out the ratio of c and d**; the present make out the determinate mixing ratio as **2:1**. The entire document of '782 patent do not teach the mixing ratio of c and d. The other cited arts do not teach this step.

9. Regarding to claim 28(a5), 31(e)

Similar as Chang et al teach and the other cited arts do not teach this step.

10. Regarding to claim 29(b1), 31(f)

Chang et al teach to inject **once a week for 4 weeks**; the present invention is: 3 hypodermic injections, each time at two weeks intervals. The number of injections and the interval is different. The '054 patent do not teach the number of injections, and the interval is different and the other cited arts do not teach this step.

11. Regarding to claim 29(b2), 31(g)

Similar as Chang et al teach. The '054 patent only teach to take eggs after hyperimmunization, do not make out the period of time and the other cited arts do not teach this step.

12. Regarding to claim 29(b3), 31(h)

The present invention teaches to take out yolk form by sieve. By using the sieve, all the egg with will go through the sieve, and the egg yolk will not be broken. So the instant invention can get the whole egg yolk, with egg white being filtered entirely, which is very important for the next steps. Further more, the filtered egg white can be used for other purpose. The cited arts fail to teach the usage of sieve. All the cited arts do not teach the usage of sieve either.

13. Regarding to claim 30(c1), 31(i)

Chang et al teach high methoxy pectin method follows by gel filtration; the present invention is water dilution, **this is the biggest difference of them.** Similar as '054 patent and Akita et al teach. The other cited arts do not teach this step.

14. Regarding to claim 30(c2), 31(j)

Similar as Chang et al, '054 patent and Akita et al teach. The other cited arts do not teach this step.

15. Regarding to claim 30(c3), 31(k)

Chang et al teach standing for 30 minutes, not **standing at 3-5°C for 20-30 hours as make out in** the present invention. Time of standing is different. Similar as '054 patent and Akita et al teach. The other cited arts do not teach this step.

16. Regarding to claim 30(c4), 31(l)

Similar as Chang et al and Akita et al teach. The '054 patent do not teach centrifugation to get supernatants, there are some complex steps instead of high-speed centrifugation. The other cited arts do not teach this step.

17. Regarding to claim 30(c5), 31(m)

Chang et al teach to filter through a No. 2 filter paper, do not teach concentrating supernatant by ultrafiltration. Similar as '054 patent and Akita et al teach. The other cited arts do not teach this step.

18. Regarding to claim 28(d), 31(n)

Chang et al teach to gel filtration with Sephacryl S-300, do not teach the use of "DEAE-Sephadex A50". The '054 patent teach purified by DEAE-SPW, DEAD-Sepharose, DEAE-Sepherodes, DEAE-650, or DE92 etc, **do not teach the use of "DEAE-Sephadex A-50" or "DEAE-Sephadex".** The other cited arts do not teach this step.

19. Regarding to claim 28(e), 31(o)

Chang et al do not teach the use of Sephadex G200. The '054 patent do not teach the use of Sephadex G200. The '376 patent teaches the use of Sephadex G200, but the subject matter of '376 patent is not to prepare IgY. It is about 'Immunological Preparations', such as 'Preparation of antigenic material/anti-B2M combination' (EXAMPLE 1), 'Preparation of antigenic material/anti-B2M/B2M/HLA combination' (EXAMPLE 2). The subject matter is different, cannot be cited as prior art. The other cited arts do not teach this step.

20. Regarding to claim 28(f), 32(p)

It is similar with claim 28(e), 31(o).

21. Regarding to claim 28(g), 32(q)

It is similar as Chang et al and Akita et al teach and the other cited arts do not teach this step.

22. Regarding to claim 28(h), 3(r)

The '094 patent teach the use of 0.22μm membrane, but the subject matter of '094 patent is to prepare **IgG** from the blood plasma of animal or human. Not to prepare **IgY.** The subject matter is different, cannot be cited as prior art. The other cited arts do not teach this step.

23. To compare the instant invention with the cited art, Chang et al, there are many differences between the present invention and the cited art, Chang et al, though Chang et al teach the 'Productivity and some properties of immunoglobulin specific against Streptococcus mutans Serotype c in chicken egg yolk'.

As shown in attachment table and the above comparisons, paragraphs (5), (6), (7), (8), (10), (12), (13), (15), (17), (18), (19), (20), (22) of the present invention are different form Chang et al teach, especially paragraph (13), Chang et al teach high methoxy pectin method, not water dilution, this is the biggest difference.

24. The applicants respectfully submit that the '782 patent teaches extracting the Streptococcus mutans antibody from milk and cultivating the Streptococcus mutans. The subject matter is different from the present invention. However, the '782 patent only teaches the preparing of S mutans, i.e. paragraphs (5), (6), (7), (8). The entire '782 patent does not teach the mixing ratio of c and d. In addition, the most important is that the subject matter of the '782 patent is to immunize cow, not hens.

25. The '054 patent suggests a LARGE-SCALE PURIFICATION OF EGG IMMUNOGLOBULIN that emphasizes on purification of IgY with a more complicated preparation procedure. It contains several times of ion exchange and process of precipitation or gel filtration and de-salting. Obviously, the cited art only discloses the PURIFICATION OF IgY, but the present invention suggests the PREPARATION OF IgY AGAINST DENTAL CARIES BACTERIA, not only the purification but also preparing streptococcus mutans antigens and immunizing hens. And in the purification of IgY, the cited art substantially differs to the present invention in many aspects as follows:

(a) Purification of IgY in the present invention only contains applying DEAE-Sephadex A50 and Sephadex G200 once. Both of the two said materials are inert substances and recyclable. No materials used in the present invention would cause chemical pollution and the cost of preparation is low. The '054 patent teaches purified by DEAE-SPW, DEAD-Sepharose, DEAE-Sepherodes, DEAE-650, or DE92 etc, but **does not teach the use of "DEAE-Sephadex A-50" and "Sephadex G200"**.

(b) In the extracting of crude IgY, the '054 patent teaches to add 1% caprylic acid in the diluted yolk when homogenize the yolk, and thus after the purification of IgY, the leavings can't be reused, and will bring circumstance pollution.

26. The Akita el al, which suggests Isolation and Purification IgY from Egg Yolk, contains steps of further purified by salt precipitation, alcohol precipitation, ultrafiltration (UF), gel filtration and anion exchange chromatography. The difference between the Akita et al and the present invention is the reagent used in the purification of IgY. The

present invention uses DEAE-Saphadex A50 and Sephadex G200. The present invention finds that the best concentrations of NaCl in phosphate buffer eluants for DEAE-Sephadex A50 column and Sephadex G200 column are 0.07M and 0.1M respectively. The IgY of present invention has reached PAGE purity with 180,000D of molecular weight by SDS-PAGE, and its activity is good.

27. The '376 patent suggests using Sephadex G-200 in purification of immunological preparations. The present invention also uses Sephadex G200, but the production of the two inventions is different, so there is no comparability between the two articles. The subject matter of '376 patent is 'Immunological Preparations', such as 'Preparation of antigenic material/anti-B2M combination' (EXAMPLE 1), 'Preparation of antigenic material/anti-B2M/B2M/HLA combination' (EXAMPLE 2), The subject matter is different, cannot be cited as prior art.

28. The subject matter of '094 patent is 'preparation of intravenous human and animal gamma globulins and isolation of albumin'. It is not about IgY. The subject matter is different, cannot be cited as prior art. In the biological field, the 0.22μm membranes is a common technology, and there is no innovation.

29. The applicants respectfully submit that the present invention must be considered as a whole and there must be something in the reference that suggests the combination or the modification. See Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick, 221 U.S.P.Q. 481,488 (Fed. Cir. 1984) ("The claimed invention must be considered as a whole, and the question is whether there is something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination"), In re Gondon, 221 U.S.P.Q. 1125, 1127 (Fed. Cir. 1984), ("The mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification.") In re Laskowski, 10 U.S.P.Q.2d 1397, 1398 (Fed.Cir. 1989), ("Although the Commissioner suggests that [the structure in the primary prior art reference] could readily be modified to form the [claimed] structure, the mere fact that the prior art could be modified would not have made the modification obvious unless the prior art suggested the desirability of the modification.")

30. In the present case, there is no such suggestion. In any case, even combining all of the said cited articles would not provide the invention as claimed – a clear indicia of nonobviousness. Ex parte Schwartz, slip op.p.5 (BPA&1 Appeal No. 92-2629 October 28,1992), ("Even if we were to agree with the examiner that it would have been obvious to combine the reference teachings in the manner proposed, the resulting package still would not comprise zipper closure material that terminates short of the end of the one edge of the product containing area, as now claimed."). That is, modifying each of the said cited articles as proposed by the Examiner, would not provide the following features:

31. **A long period of validity:** In the claimed invention, after first immunize hens 20th days, eggs can keep active antibody (immunoglobulin of yolk) for approximately 13 months. In current documents, this duration of existing techniques is only half a year. So, the period of validity increases two times than the current techniques.

32. **High titer of antibody:** If existing preparative techniques are used to prepare antibody, the highest titer is only 1:320 at present. In the present invention, the titer can be 1:512. More antibodies can be produced in the present invention in the same circumstance.

33. **Good effect on restraining activity of streptococcus mutans :** Research has showed that forty-eight hours after different concentrations of IgY are put into culture medium of streptococcus mutans, activity of streptococcus mutans is restrained, and the pH rises to different extents. This result proves that every pH is above 6.0 (Experiment proves dental caries occur only at the critical value pH 5.0~5.5 or even below). And animal experiment also further suggested that IgY can effectively protect dental caries.

34. **No chemical pollution and comprehensively utility of remains:** There is no chemical pollution in the reparation process. After extracting, the eggshell can be made into calcium powder, egg white can be made into peptone, the remainder of yolk can be made into food, or be utilized as lecithin, vitalize oil and other food. There leaves on cast off, therefore there is no environmental pollution.

35. **Low preparation cost:** In both processes of extracting crude IgY and purifying extracted IgY, the techniques are very simple and DEAE-Sephadex A50 and Sephadex

G200 can be utilized repeatedly. Therefore, the whole preparation cost is relatively lower than current techniques.

36. **Implementation probability:** The success ration of implementing the present invention will reach 100% if the implementation strictly follows the operation of the present invention.

37. **High purity**: Follow the steps of the present invention; especially using the DEAE-Sephadex A50 and Sephadex G200 can make the IgY of the present invention reach PAGE purity with 180,000D of molecular weight by SDS-PAGE. The best concentrations of NaCl in phosphate buffer eluates for DEAE-Sephadex A50 column and Sephadex G200 column are 0.07M and 0.1M respectively. In other words, the present invention obtains above better purity because of selecting proper concentration as described above.

(i) **Dental caries-preventing combinations with IgY:** as recited in claim 33, the IgY of the present invention can be used as a effective component to prepare combination against dental caries bacteria. The using of potassium sorbate and/or sodium benzoate will maintain the activity of IgY, and make sure the IgY will not be polluted by other bacterial.

(ii) **IgY productions:** The IgY of the present invention can makeup many different kinds of IgY productions, such as IgY buccal liquid, IgY chewing gum, IgY toothpaste, IgY tooth-protecting paste, IgY nutrient milk, IgY nutrient milk powder or IgY nutrient bean milk et al.

The Cited but Non-Applied References

38. The cited but not relied upon references have been studied and are greatly appreciated, but are deemed to be less relevant than the relied upon references.

39. In view of the above, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of the rejection are requested. Allowance of claims 28 to 33 at an early date is solicited.

40. Should the Examiner believe that anything further is needed in order to place the application in condition for allowance, he is requested to contact the undersigned at the telephone number listed below.

Respectfully submitted,

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CERTIFICATE OF MAILING

I hereby certify that this corresponding is being deposited with the United States Postal Service by First Class Mail, with sufficient postage, in an envelope addressed to "Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450" on the date below.

Date: May 19, 2003

Signature:
Person Signing: Raymond Y. Chan

Table 1 One by one comparisons (the claims of the present invention VS cited art)

	Claim	our application	CHANG et al		782 PATENT		054 PATENT		AKITA et al		
			teach	differ	teach	differ	teach	differ	teach	differ	
1	28(a1), 31(a)	cultivating S mutans type c and d for 2 to 3 days	cultivating c and a, b, d, e, f for 18 hours	cultivating time is different.	cultivating S. mutans for 48 hours,	782 patent do not teach the cultivating of c, d		do not teach		do not teach	d
2	28(a2), 31(b)	collecting bacteria by centrifugation	treat with 0.5% formalin for 24 h,	the present invention need not to treat with formalin	collecting bacteria by centrifugation	similar		do not teach		do not teach	s
3	28(a3), 31(c)	washing 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes	washing with sterile saline containing 05.% formalin	the phosphate buffered saline of the present invention containing no formalin	washing 4 to 6 times with phosphate saline solution, and heating at 56°C for 2 hours, or at 60°C for 30 minutes	similar		do not teach		do not teach	s
4	28(a4), 31(d)	mixing c and d in a ratio of 2:1	70% caused by c, c and d applied simultaneously	Chang et al do not teach the mixing ratio of c and d, especial the ratio of d		the entire document of '782 patent do not teach the mixing ratio of c and d		do not teach		do not teach	d
5	28(a5), 31(e)	adjuvant ,high speed homogenized	adjuvant ,homogenized	similar		do not teach		do not teach		do not teach	s
6	29(b1), 31(f)	three injections, each time at two weeks intervals	once a week for 4 weeks	the number of injections and the interval is different		do not teach	two weeks intervals	054 do not teach the number of injections, and the interval is different		do not teach	d
7	29(b2), 31(g)	collecting eggs from 20th day after first hypodermic injection	collecting eggs over 4-10 weeks	similar		do not teach	after hyperimmunization	054 do not make out the period of time		do not teach	s

8	29(b3), 31(h)	taking out yolks from eggs by sieve	not teach	using the sieve, egg white will go through, and then we can get the whole yolk. Prior art did not teach the usage of sieve.		do not teach		do not teach		do not teach	d
9	30(c1), 31(i)	stirring yolks and diluting with 4-6 fold of distilled water to obtain a diluted yolk solution	high methoxy pectin method follows by gel filtration	high methoxy pectin method , not water dilution, ***this is the biggest difference***		do not teach	water dilution with 4-6 fold of distilled water	similar	water dilution with 6 fold of water	similar	s
10	30(c2), 31(j)	adjusting to pH 4.5-6.5	adjusting to pH 5	similar		do not teach	adjusting to pH 4-6	similar	adjusting to pH 5.0-5.2	similar	s
11	30(c3), 31(k)	standing at 3-5°C for 20-30 hours	standing for 30 minutes	time of standing is different		do not teach	standing at 3-4°C for at least 2 hours	similar	stand for at least 2 hours, 6hours at 4°C	similar	s
12	30(c4), 31(l)	centrifuging for 20-30 minutes to obtain supernatant	centrifugation to get supernatants	similar		do not teach	get immunoglobulins in the bottom aqueous layer,	054 do not teach centrifugation to get supernatants, there is ***no supernatant***	centrifuging at high speed to obtain supernatant	similar	s
13	30(c5), 31(m)	concentrating supernatant by ultrafiltration, eliminating bacteria and lyophilization to achieve crude IgY	filter through a No. 2 filter paper	Chang et al do not teach concentrating supernatant by ultrafiltration		do not teach	concentrating supernatant by ultrafiltration, get crude IgY	similar	concentrating supernatant by ultrafiltration	similar	s
14	28(d), 31(n)	applying crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer to obtain eluates of protein peak	gel filtration with Sephacryl S-300	the difference is obvious		do not teach	purified by DEAE-SPW, DEAD-Sepharose, DEAE-Sepherodes, DEAE-650, DE92 etc,	054 do not teach the use of DEAE-Sephadex		do not teach	

15	28(e), 31(o)	applying eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain a new eluates of protein peak		Chang et al do not teach		do not teach	Sephadex column chromatography	054 do not teach the use of Sephadex G200		do not teach	d
16	28(f), 32(p),	collecting new eluates of protein peak								do not teach	d
17	28(g), 32q),	estimating antibody activity of protein peaks with "ELISA"	activity of S. mutans was determined by ELISA	similar		do not teach		do not teach		do not teach	s
18	28(h), 32(r),	eliminating bacteria by 0.22μm membrane filtration and lyophilizing to achieve a purified IgY against dental caries bacteria		Chang et al do not teach		do not teach		do not teach		do not teach	d